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(71)出願人　000002141
住友ベークライト株式会社
東京都品川区東品川２丁目５番８号
(72)発明者　田中　速雄
秋田市土崎港相染町字中島下27－４　秋田住友ベーク株式会社内

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(54)【発明の名称】　蛋白質分析用微小回路

(57)【要約】
【課題】　回路壁面への蛋白質の吸着制御及び回路表面の平滑性の向上させることによる感度、精度、再現性を向上させる蛋白質分析用微小回路の提供する。
【解決手段】　回路表面への超親水性ポリマーのコーティングにより、蛋白質の吸着制御及び平滑性を向上させる。

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【特許請求の範囲】

【請求項１】　蛋白質溶液が接触する断面積１ｍｍ^{2}以下の微小回路において、回路の少なくとも蛋白質溶液と接触する表面が超親水性ポリマーで被覆されている蛋白質分析用微小回路。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、蛋白質の構造・機能解析及び蛋白質を使用した反応に用いられる装置・機器の内、蛋白質溶液の流動または反応または分析を行なう微小回路に関する。

【０００２】

【従来の技術】現在、種々の化学反応を行なう為の微小回路は反応効率、速度、省試薬の観点から注目されつつある技術であり、既に「ラボオンアチップ」と呼ばれる数センチ角の硝子製チップ上に形成された回路の中で化学反応・分析を行なう新しい分析方法に関する概念が一般的に定着している。今後、バイオテクノロジーの進展に伴い生化学分野においても微小回路の利用は不可欠な技術であり、特に蛋白質の構造・機能解析及び蛋白質を使用した反応への微小回路の応用が期待されている。

【０００３】蛋白質溶液を微小回路に流す際に大きな問題となるのは回路表面への蛋白質の吸着であり、微量蛋白質は吸着による減少及び構造変化の影響を大きく受けるだけでなく、回路をくり返し使用する場合には吸着した蛋白質が履歴として残る点、更に回路自体が微小であるために吸着残留した蛋白質によって回路が閉塞する恐れのある点も問題となる。また微小回路内で２種類の溶液を流動させた状態で反応を行なう場合には、２種類の溶液が１本の回路内で相流を形成する事が重要であるが、吸着残留した蛋白質によって回路壁面に凹凸が形成され、その結果乱流が発生し反応効率が低下してしまう点は微小回路にとって非常に大きな問題である。

【０００４】現在微小回路に使用されている基材の殆どは硝子（石英硝子）若しくはプラスチック類であるが、それらの基材に対して蛋白質は高い吸着性を示す。また微小回路の加工性に関する問題点として、基材上に微小回路を形成した際に表面に微細な凹凸が発生する場合があり、その様な凹凸は前述の理由により好ましくない。

【０００５】

【発明が解決しようとする課題】本発明の目的は感度、精度、再現性を向上させる蛋白質分析用微小回路の提供であり、回路壁面への蛋白質の吸着及び回路表面の平滑性の向上が前記目的を達成する為の課題であると考えた。

【０００６】

【課題を解決するための手段】即ち本発明は、蛋白質溶液が接触する断面積１ｍｍ^{2}以下の微小回路において、回路の少なくとも蛋白質溶液と接触する表面が超親水性ポリマーで被覆されている蛋白質分析用微小回路である。

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【０００７】

【発明の実施の形態】従来の硝子又はプラスチックへの蛋白質吸着は短時間の接触で発生し、低濃度領域（約１ｎｇ～１００ｕｇ／ｍｌ）においてその吸着率（接触させた蛋白質溶液中の蛋白質の内吸着する蛋白質の割合）は、最大約５０％にも達し、一度吸着した蛋白質は不可逆な構造変化（変性）を起こし、変性した蛋白質は二次的な蛋白質の吸着を誘引し、結果として蛋白質の多層吸着層が形成される。

【０００８】そこで、本発明においては蛋白質溶液が接触する表面を超親水性ポリマーで被覆する事によって、蛋白質の吸着を引き起こす最も大きな要因である疎水性相互作用を低減し、蛋白質の初期の吸着を防止している。超親水性ポリマーとは水との親和性に非常に優れたポリマーで、水に浸漬する事により表面に均一な自由水層を保持し、その水接触角が０～１度となる高分子材料を指す。

【０００９】超親水性ポリマーの例としては、ポリヒドロキシアルキルメタクリレート、ポリオキシＣ２－Ｃ４アルキレン基含有メタクリレート重合体又はこれを含む共重合体、あるいはポリビニルピロリドン、リン脂質・高分子複合体（特開平５－１６１４９１号公報及び特開平６－４６８３１号公報）、２－メタクリロイルオキシエチルホスホリルコリン共重合体（以下ＭＰＣと略す）又はこれを含む共重合体（特開平９－３１３２号公報）などが挙げられる。

【００１０】超親水性ポリマーの被覆は蛋白質の吸着防止以外の効果も有している。超親水性ポリマーは液体と接触する事で自由水を含み膨潤し、その表面は平滑になる。すなわち、回路表面の乱流の原因となる加工時の凹凸が超親水性ポリマーの被覆及び膨潤により低減される。超親水性ポリマーを被覆する際に注意すべき点は被覆層の厚みである。超親水性ポリマーの被覆層が厚すぎると、蛋白質溶液と接触して膨潤した際に回路を閉塞してしまう可能性があり、また、蛋白質の吸着性能も低下するため被覆層の厚みは５ｕｍ以下が好ましく、表面が完全に覆われていれば可能な限り薄い層であることが好ましい。

【００１１】超親水性ポリマーを被覆する方法としては特に限定するものでは無いが、超親水性ポリマーを溶媒に溶解した溶液を回路内に充填し、回路の開口端に吸引ポンプに接続した吸引ノズルをあて、充填した親水性ポリマー溶液を吸引し、回路表面に残留した親水性ポリマーが乾燥するまで吸引を続ける方法が好ましい。前記被覆方法によって、回路が閉塞する事無く超親水性の被覆層が形成され、その厚みは親水性ポリマー溶液の粘度によって容易に調節する事が出来る。また、エアレーション乾燥によって平滑な被覆表面を得る事が出来る。以下、実施例によって本発明を更に具体的に説明する。

【００１２】
【実施例】（実施例１）厚さ２mm、２０×３０mm角のポリスチレンプレートの長軸方向にドリルを用いて直径０．５mm、長さ３０mmの直線状の回路を形成した。回路の一方の開口端からポリヒドロキシエチルメタクリレート（ＳＩＧＭＡ製Ｐ－３９３２）の２．５Ｗｔ／ｖｏｌ％メタノール溶液を注入した。注入の際、０．５mmφの鈍針を先端に取り付けた２．５ｍｌのシリンジを使用した。注入後開口端にテープでふたをして３０分間静置した後に、吸引ポンプートラップに接続したノズルを開口端に押し当て、１分間吸引を行なった。更に一晩乾燥させた。乾燥後のコート層の厚みを回路部分を切り出して電子顕微鏡にて測定したところ、厚みは約１．３umであった。
【００１３】（実施例２）実施例１で使用した物と同一の回路の一方の開口端からＭＰＣポリマーの０．５ｗｔ／ｖｏｌ％エタノール溶液を注入した。ＭＰＣポリマーは、「リン脂質類似構造を有するハイドロゲル膜からの薬物放出　高分子論文集，４６，５９１－５９５（１９８９）」の内容に従いＭＰＣとＢＭＡ（ブチルメタクリレート）比＝３／７の共重合体を合成し使用した。注入後開口端にテープでふたをして３０分間静置した後に、吸引ポンプートラップに接続したノズルを開口端に押し当て、１分間吸引を行なった。更に一晩乾燥させ、実施例２とした。乾燥後のコート層の厚みを回路部分を切り出して電子顕微鏡にて測定したところ、厚みは約０．５umであった。
【００１４】（比較例１）厚さ２mm、２０×３０mm角のポリスチレンプレートの長軸方向にドリルを用いて直径０．５mm、長さ３０mmの直線状の回路を形成したものを比較例１とした。
【００１５】（比較例２）厚さ２mm、２０×３０mm角のガラス製プレートの長軸方向にドリルを用いて直径０．５mm、長さ３０mmの直線状の回路を形成したものを比較例２とした。
【００１６】（蛋白質吸着性の比較）実施例１、実施例２及び比較例１、比較例２の回路部分に５０ｎｇ／ｍｌのウシアルブミン（ＢＳＡ）溶液２００ｕｌをくり返し３０回循環させた後にＢＳＡ溶液の濃度を測定し、濃度の変化率を求めた。ＢＳＡ溶液の濃度の測定は以下の手順で実施した。
【００１７】回収したＢＳＡ溶液をＥＬＩＳＡ用プレート（住友ベークライト製　スミロンＥＬＩＳＡ用プレートH）に分注し、３７℃で１時間インキュベート。その後プレートウォッシャーを用いて３回洗浄を繰り返した。尚、洗浄液には０．０５％Ｔｗｅｅｎ２０含有リン酸緩衝液（日水製薬製　ダルベッコＰＢＳ－ｐＨ７．４）を使用した。
【００１８】その後、３％スキムミルク（コスモバイオ製）リン酸緩衝液溶液を２５０μＬ／ウェルで分注し、３７℃で１時間インキュベートした。その後プレートウォッシャーを用いて３回洗浄を繰り返した。次にペルオキシターゼ標識抗ウシアルブミン抗体（コスモバイオ製）の１．５ｕｇ／ｍｌリン酸緩衝液溶液を１００ｕｌ／ウェルで分注し、室温で３０分静置した後、プレートウォッシャーを用いて３回洗浄を繰り返した。
【００１９】次にＴＭＢＺ基質緩衝液（住友ベークライト製　スミロンペルオキシターゼ用発色キットＴ）を用いて発色させた後に、プレートリーダーにて吸光度を測定、検量線から濃度を求め、初期濃度からの変化率を求めた。結果は表１の通りで、実施例１、実施例２共に比較例１、比較例２に比べて蛋白質溶液中の蛋白質の濃度変化が大幅に抑えられている事を確認した。
【００２０】
【表１】

試料	濃度変化率
実施例１	１．０％
実施例２	１．４％
比較例１	４１．７％
比較例２	３３．６％

【００２１】（回路閉塞性の比較）回路に吸着残留した蛋白質が与える影響を比較する為に、実施例１、比較例１を用いて以下の検討を実施した。実施例１、比較例１各々の回路の開口端にシリコン製チューブを接着接続し、その中に５００ｍｇ／ｍｌのＢＳＡリン酸緩衝液溶液を充填し、そのチューブをペリスターポンプに接続する事でＢＳＡ溶液を回路に連続的に循環させる事の出来る装置を作製した。
【００２２】実施例１、比較例１各々に接続したペリスターポンプを１時間稼動させた後に純水洗浄後乾燥、更にポンプに接続した後にＢＳＡ溶液を１時間循環させる作業を５日間（３０回）くり返し実施した後の回路の内腔面の状態を観察した。その結果、実施例１においてはＢＳＡの吸着層は確認されず回路内腔面は検討開始時の状態を保持していたが比較例１においては内腔面に一様にＢＳＡが付着しており、所々で瘤状のかたまりが確認され、回路内でのＢＳＡ溶液の流れが妨げられている事は明らかであった。
【００２３】
【発明の効果】以上述べた如く、本発明によれば下記の優れた効果が得られる。第一に、回路表面への蛋白質の吸着が無く、分析を行なう蛋白質の減少及び変性が発生しないために、微量かつ高精度な分析が可能となる。第二に、回路表面への蛋白質の吸着が無く、使用した蛋白質が回路内に履歴として残る事無く、くり返し精度の優れた分析を行なう事ができる。第三に、回路表面への蛋白質の吸着が無く、回路内の乱流の発生が抑えられ閉塞の問題も無い。第四に、超親水性ポリマーのコーティン

グ及び膨潤により回路表面の平滑性向上も期待される。

PATENT ABSTRACTS OF JAPAN

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(71)Applicant : SUMITOMO BAKELITE CO LTD
(72)Inventor : TANAKA HAYAO

(54) MICROCIRCUIT FOR ANALYZING PROTEIN

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a microcircuit for analyzing protein capable of improving sensitivity, accuracy and reproducibility by improving the adsorption control of protein to a circuit wall face and the smoothness of a circuit surface.
SOLUTION: The control of the adsorption of protein and the smoothness can be improved by coating the circuit surface with super-hydrophilic polymer.

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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] Microcircuit for protein analyses where the front face of a circuit which contacts a protein solution at least is covered with the super-hydrophilic-property polymer in the with a 1mm cross section [or less 2] microcircuit where a protein solution contacts.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the microcircuit which performs a flow, a reaction, or analysis of a protein solution among the equipment and the device used for the reaction which used proteinic structure and functional analysis, and protein.
[0002]
[Description of the Prior Art] The microcircuits for performing current and various chemical reactions are reaction effectiveness, a rate, and a technique that is attracting attention from a viewpoint of ******, and, generally the concept about the new analytical method which performs a chemical reaction and analysis in the circuit formed on the chip made from glass of several cm angle already called a "lab ONA chip" is established. From now on, with progress of biotechnology, also in the biochemistry field, use of microcircuit is an indispensable technique and application of the microcircuit to the reaction which used proteinic structure and functional analysis, and protein especially will be expected.
[0003] The point that the protein to which it stuck when it is not only greatly influenced of reduction and a structural change according [in case a protein solution is poured to microcircuit, adsorption of the protein on the front face of a circuit poses a big problem, and / minute amount protein] to adsorption, but a circuit was repeated and used remains as hysteresis, and a point with a possibility that a circuit may blockade with the protein which carried out the adsorption residual since the circuit itself was still minuter also pose a problem. Moreover, although it is important that two kinds of solutions form a phase style in one circuit when reacting in the condition of having made two kinds of solutions flowing in microcircuit, the point that irregularity is formed in a circuit wall surface, a turbulent flow occurs as a result, and reaction effectiveness falls with the protein which carried out the adsorption residual is a very big problem for microcircuit.
[0004] Although most base materials currently used for current microcircuit are glass (silica glass) or plastics, protein shows adsorbent [high] to those base materials. Moreover, as a trouble about the workability of microcircuit, when microcircuit is formed on a base material, irregularity detailed on a front face may occur and such irregularity is not desirable by the above-mentioned reason.
[0005]
[Problem(s) to be Solved by the Invention] I thought that the purpose of this invention was offer of the microcircuit for protein analyses which raises sensibility, precision, and repeatability, and was a technical problem for adsorption of the protein to a circuit wall surface and improvement in the smooth nature on the front face of a circuit to attain said purpose.
[0006]
[Means for Solving the Problem] That is, this invention is microcircuit for protein analyses where the front face of a circuit which contacts a protein solution at least is covered with the super-hydrophilic-property polymer in the with a 1mm cross section [or less 2] microcircuit where a protein solution contacts.
[0007]
[Embodiment of the Invention] The protein adsorption to conventional glass or plastics is generated in short-time contact. In a low concentration field (about 1ng- 100 ug/ml) the surface coverage (protein to which the protein in the contacted protein solution inner-sticks comparatively) About 50% of maxes is also reached, in the structural change (denaturation) with the irreversible protein to which it stuck once, adsorption of protein with secondary lifting and denatured protein is attracted, and a proteinic multilayer adsorption layer is formed as a result.
[0008] Then, by covering with a super-hydrophilic-property polymer the front face where a protein solution contacts in this invention, the hydrophobic interaction which is the biggest factor that causes adsorption of protein was reduced, and the adsorption in early stages of proteinic is prevented. A super-hydrophilic-property polymer is a polymer which was very excellent in compatibility with water, by being immersed in water, holds a uniform free water layer on a front face, and points out the polymeric materials from which the water contact angle becomes 0 - 1 time.
[0009] The copolymer (JP,9-3132,A) which contains the copolymer containing polyhydroxy alkyl methacrylate, a polyoxy C2-C4 alkylene-group content methacrylate polymer, or this or a polyvinyl pyrrolidone, phospholipid and macromolecule complex (JP,5-161491,A and JP,6-46831,A), 2-methacryloiloxy-ethyl phosphorylcholine copolymer (it omits Following MPC), or this as an example of a super-hydrophilic-property polymer is mentioned.
[0010] Covering of a super-hydrophilic-property polymer also has effectiveness other than adsorption prevention of

protein. Swelling a super-hydrophilic-property polymer including free water by contacting a liquid, the front face becomes smooth. That is, the irregularity at the time of processing leading to the turbulent flow on the front face of a circuit is reduced by covering and swelling of a super-hydrophilic-property polymer. The point which it should be careful of in case a super-hydrophilic-property polymer is covered is the thickness of an enveloping layer. When the enveloping layer of a super-hydrophilic-property polymer is too thick, since a circuit may be blockaded and the proteinic adsorption engine performance also falls when a protein solution is contacted and it swells, the thickness of an enveloping layer has 5 or less desirable ums, and if the front face is covered completely, it is desirable that it is a film as much as possible.

[0011] The approach of continuing suction is desirable until the hydrophilic polymer which was filled up with the solution which dissolved the super-hydrophilic-property polymer in the solvent in the circuit, attracted the hydrophilic polymer solution hit and filled up with the suction nozzle which connected with the suction pump at the opening edge of a circuit, and remained on the circuit front face although there was nothing what is limited especially as an approach of covering a super-hydrophilic-property polymer dries. The enveloping layer of a super-hydrophilic property is formed by said covering approach, without a circuit blockading, and the thickness can be easily adjusted with the viscosity of a hydrophilic polymer solution. Moreover, a smooth covering front face can be obtained by aeration desiccation. Hereafter, an example explains this invention still more concretely.

[0012]

[Example] (Example 1) The drill was used in the direction of a major axis of the polystyrene plate of 2mm in thickness, and 20x30mm angle, and the circuit of the shape of a straight line with a diameter [of 0.5mm] and a die length of 30mm was formed in it. The 2.5 Wt/vol% methanol solution of polyhydroxyethyl methacrylate (product P-3932 made from SIGMA) was poured in from one opening edge of a circuit. The 2.5ml syringe which attached stumpfe N of 0.5mmphi at the tip was used at the time of impregnation. After covering the opening edge after impregnation and putting it on it gently for 30 minutes on a tape, the nozzle linked to a suction pump-trap was pressed against the opening edge, and suction was performed for 1 minute. Furthermore, it was made to dry overnight. When the circuit part was started and the thickness of the coat layer after desiccation was measured with the electron microscope, thickness was about 1.3 um(s).

[0013] (Example 2) The 0.5 wt/vol% ethanol solution of an MPC polymer was poured in from one opening edge of the same circuit as the object used in the example 1. an MPC polymer — the contents of "the drug release giant-molecule collected works from the hydro gel film which has phospholipid similar structure and 46,591-595 (1989)" — following — MPC and a BMA (butyl methacrylate) ratio — the copolymer of =3/7 was compounded and used. After covering the opening edge after impregnation and putting it on it gently for 30 minutes on a tape, the nozzle linked to a suction pump-trap was pressed against the opening edge, and suction was performed for 1 minute. Furthermore, it was made to dry overnight and considered as the example 2. When the circuit part was started and the thickness of the coat layer after desiccation was measured with the electron microscope, thickness was about 0.5 um(s).

[0014] (Example 1 of a comparison) What used the drill in the direction of a major axis of the polystyrene plate of 2mm in thickness and 20x30mm angle, and formed the circuit of the shape of a straight line with a diameter [of 0.5mm] and a die length of 30mm in it was made into the example 1 of a comparison.

[0015] (Example 2 of a comparison) What used the drill in the direction of a major axis of the glass plate of 2mm in thickness and 20x30mm angle, and formed the circuit of the shape of a straight line with a diameter [of 0.5mm] and a die length of 30mm in it was made into the example 2 of a comparison.

[0016] (Comparison of protein adsorption nature) After repeating 50 ng(s)/ml bovine albumin (BSA) solution 200ul into the circuit parts of an example 1, an example 2 and the example 1 of a comparison, and the example 2 of a comparison and circulating them 30 times, the concentration of a BSA solution was measured, and it asked for the rate of change of concentration. Measurement of the concentration of a BSA solution was carried out in the following procedures.

[0017] The collected BSA solution is poured distributively on the plate for ELISA (the plate H for Sumitomo Bakelite SUMIRON ELISA), and it incubates at 37 degrees C for 1 hour. Washing was repeated 3 times using the plate washer after that. In addition, the Tween20 content phosphate buffer solution (NISSUI PHARMACEUTICAL Dulbecco PBS-pH7.4) was used for the penetrant remover 0.05%.

[0018] Then, the skim milk (Cosmobio make) phosphate buffer solution solution was poured distributively by 250microL / well 3%, and it incubated at 37 degrees C for 1 hour. Washing was repeated 3 times using the plate washer after that. Next, after pouring distributively 1.5 ug(s)/ml phosphate buffer solution solution of a peroxidase indicator pit bovine albumin antibody (Cosmobio make) by 100ul(s) / well and putting it at a room temperature for 30 minutes, washing was repeated 3 times using the plate washer.

[0019] Next, after making it color using the TMBZ substrate buffer solution (the coloring kit T for Sumitomo Bakelite SUMIRON peroxidase), the absorbance was asked for concentration from measurement and a calibration curve with the plate reader, and it asked for the rate of change from initial concentration. A result is as in Table 1 and, as for the example 1 and the example 2, concentration change of the protein in a protein solution checked being stopped sharply compared with the example 1 of a comparison, and the example 2 of a comparison.

[0020]

[Table 1]

試料	濃度変化率
実施例 1	1．0％
実施例 2	1．4％
比較例 1	41．7％
比較例 2	33．6％

[0021] (Circuit obstructive comparison) In order to compare the effect which the protein which carried out the adsorption residual has on a circuit, the example 1 and the example 1 of a comparison were used, and the following examination was carried out. an example 1 and the example 1 of a comparison — adhesion connection of the tube made from silicon was made at the opening edge of each circuit, it was filled up with the 500mg [/ml] BSA phosphate buffer solution solution into it, and the equipment which can make a circuit circulate through a BSA solution continuously by connecting the tube to a ** RISUTA pump was produced.
[0022] The condition of the lumen side of the circuit after repeating the activity which it circulates through the desiccation after pure-water washing after working the ** RISUTA pump linked to example 1 and example of comparison 1 each for 1 hour, and circulates a BSA solution for 1 hour after connecting with a pump further for five days (30 times) and carrying it out was observed. Consequently, it was clear that the adsorption layer of BSA was not checked in the example 1, but BSA has adhered to the lumen side uniformly in the example 1 of a comparison although the circuit lumen side held the condition at the time of examination initiation, a phyma-like lump is checked in some places, and the flow of the BSA solution in a circuit is barred.
[0023]
[Effect of the Invention] As stated above, according to this invention, the effectiveness which was excellent in the following is acquired. In the first place, there is no adsorption of the protein on the front face of a circuit, and since a proteinic reduction and the denaturation which analyze do not occur, a minute amount and highly precise analysis are attained. Analysis which was excellent in repetition precision can be performed without there being no adsorption of the protein on the front face of a circuit in the second, and the used protein remaining as hysteresis in a circuit. There is no adsorption of the protein on the front face of a circuit in the third, generating of the turbulent flow in a circuit is suppressed and there is also no problem of lock out. The smooth disposition top on the front face of a circuit is also expected from the fourth by coating and swelling of a super-hydrophilic-property polymer.

[Translation done.]